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Regular exercise training reverses ectonucleotidase alterations and reduces hyperaggregation of platelets in metabolic syndrome patients

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ABSTRACT

Background: Alterations in the activity of ectonucleotidase enzymes have been implicated in cardiovascular diseases, whereas regular exercise training has been shown to prevent these alterations. However, nothing is known about it relating to metabolic syndrome (MetS). We investigated the effect of exercise training on platelet ectonucleotidase enzymes and on the aggregation profile of MetS patients.
Methods: We studied 38 MetS patients who performed regular concurrent exercise training for 30 weeks. Anthropometric measurements, biochemical profiles, hydrolysis of adenine nucleotides in platelets and platelet aggregation were collected from patients before and after the exercise intervention as well as from individuals of the control group.
Results: An increase in the hydrolysis of adenine nucleotides (ATP, ADP and AMP) and a decrease in adenosine deamination in the platelets of MetS patients before the exercise intervention were observed ($P < 0.001$). However, these alterations were reversed by exercise training ($P < 0.001$). Additionally, an increase in platelet aggregation was observed in the MetS patients ($P < 0.001$) and the exercise training prevented platelet hyperaggregation in addition to decrease the classic cardiovascular risks.
Conclusions: An alteration of ectonucleotidase enzymes occurs during MetS, whereas regular exercise training had a protective effect on these enzymes and on platelet aggregation.

1. Introduction

Lifestyles characterized by excessive calories intake and low practice of physical exercise are responsible for the high prevalence of metabolic syndrome (MetS), which represents a collection of cardiovascular risk factors: hypertension, hyperglycemia, dyslipidemia and abdominal obesity [1,2]. More than 312 million adults worldwide have been diagnosed with MetS and it is estimated that this number will rise to 1 billion by 2025. In Brazil, approximately 29.6% of the population have MetS and it is considered a public health problem [3].

Current treatments of MetS do not consider the cardiovascular risk factor associations, which are a greater damage than each risk factor alone by favoring pro-thrombotic and pro-inflammatory states [4]. Indeed, purinergic system, that is involved in modulation of inflammatory and thromboembolic processes, has been shown altered in cardiovascular diseases (CVD). Infarcted patients showed alterations in the activity of ectonucleotidase enzymes in platelets [5], as well as patients with acute coronary syndrome, hypertension and diabetes [6–8]. However, the effects of MetS on purinergic enzymes are still unknown.

Ectonucleotidase enzymes are expressed on surface of several cells, including platelets, to hydrolyze extracellular nucleotides (ATP, ADP and AMP) and then controlling their levels. This enzymatic complex includes the enzymes E-NTPDase (ectonucleoside triphosphate diphosphohydrolase), E-NPP (ectonucleotide pyrophosphatase/phosphodiesterase), E-5′-nucleotidase and adenosine deaminase (ADA) [9]. E-NTPDase hydrolyzes ATP and ADP in AMP [10], whereas the E-NPPs hydrolyze 5′-phosphodiester bonds in nucleotides and their derivatives to produce nucleotide monophosphate [11]. AMP, resulting from the actions of E-NTPDase and E-NPP, is subsequently hydrolyzed into adenosine by E-5′-nucleotidase [12].

Additionally, adenosine can be directly inactivated on the cell surface through the sequential actions of ADA, which catalyzes the irreversible deamination of adenosine and leads to inosine [13]. Together, these ecto-enzymes constitute a highly organized enzymatic cascade capable of regulating the extracellular concentrations of adenine

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nucleotides and nucleoside. This cascade plays an important role in the maintenance of normal homeostasis and thrombogenesis by regulating platelet aggregation [14]. It is noteworthy that micromolar concentrations of ADP can act in platelet P_2Y_{12} receptors and induce human platelet aggregation, whereas adenosine (the final product of ATP hydrolysis) can inhibit platelet aggregation [15].

Recent studies have demonstrated a potential effect of the physical exercise in reversing purinergic system alterations during CVD [16,17], while epidemiological and clinical studies have demonstrated that regular physical exercise is an important factor to the prevention and treatment of MetS [18–20]. Concurrent training, which combines aerobic and strength exercises in the same training session, has shown benefits in insulin action, endothelial function, lean body mass, and glycemic control (especially when the exercise is performed in moderate intensity and regularly) [19]. Therefore, concurrent training can act on all the cardiovascular risk factors simultaneously and can be a powerful tool to improve the quality of life in patients with MetS [21]. Besides, it was observed in animal models that the exercise training induces a downregulation in the activity of ectonucleotidase enzymes, which results in a protective effect against hypertension [16]. However, the effect of exercise training on ectonucleotidase activities in patients with MetS is still unknown.

Taking into account that exercise training exerts a modulating effect on ectonucleotidase activities and that we hypothesized that ectonucleotidase activities can be altered in the platelets of MetS patients, including impairment of vascular homeostasis, we investigated the effect of 30 weeks of regular concurrent training on the activity of enzymes that hydrolyze adenine nucleotides and nucleoside in the platelets of patients with MetS. Exercise training is widely known to benefit the cardiovascular system. However, studying the association between the activity of ectonucleotidases in MetS patients and the influence of exercise on these enzymatic activities can be a source of information for producing new treatments for MetS.

2. Patients and methods

2.1. Chemicals

Nucleotides, sodium azide, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), and the Trizma base were purchased from Sigma-Aldrich. All other reagents used in the experiments were of analytical grade and highest purity.

2.2. Metabolic syndrome patients

Thirty-eight volunteer MetS patients from the study group at the Center of Physical Education at the Federal University of Santa Maria were selected for this study. The MetS patients were recruited through a single-stage, using a random sampling with ages between 55 and 65 y and a representative number of both sexes. MetS was characterized according to the classification of the National Cholesterol Education Program — Adult Treatment Panel III (NCEP — ATP III, 2001) [22].

All subjects signed the consent to participate in the study. The protocol was approved by the Human Ethics Committee of the Health Science Center at the Federal University of Santa Maria, protocol number 19435813.8.0000.5346, Brazil.

The first collection of samples was carried out during the diagnosis of MetS patients, which constituted the MetS pre-intervention measurements. Then, the MetS group conducted the exercise intervention for thirty weeks. Approximately 24 h after the last exercise session, the second collection of samples was carried out, which constituted the MetS post-intervention measurements. Ten milliliters of peripheral blood were collected by venipuncture from each patient pre- and post-intervention to isolate platelets and obtaining serum.

2.3. Exercise intervention

The exercise plan proposed for the MetS patients was concurrent and moderate with aerobic and resistance exercises. The supervised training took place in the Physical Education and Sports Center gym of the Federal University of Santa Maria. MetS patients initially received a training adaptation for the exercises with a low intensity indoor walking for 10 min and resistance training with few exercises and low volume and intensity for familiarization of movement performance technique. After one week of adaptations, the volunteers performed the exercise training during 30 weeks, and the activities were performed three times a week totaling 90 sessions. Volume and intensity of training were progressive to avoid adaptations to stimuli caused by exercise and a good technique practice was emphasized, reducing the potential for excessive muscle soreness and injury.

For aerobic training, the volunteers were instructed to walk on a moderate rate for about 30 min in the first weeks. After, they increased the walk intensity over the months to about 45 min with relatively fast speed.

In resistance training, the practice initially was performed with only 4 different types of exercises. Every 2 weeks, a different exercise was added to the plan totaling 11 types of exercises: chest press, rower machine, lat pull-down, triceps pulley extension, biceps curl, leg press, leg curl, ankle plantar flexion, hip abduction, hip adduction and abdominals. The MetS patients performed all exercises in three sets of ten repetitions with an interval of 1′ to 1′30″ between sets and 2′ to 3′ between exercises, alternating upper and lower resistance machines. The intensity of the work was set at 70% of the maximum force, which was calculated for each patient using the test of the repetition maximum [23]. This test is used to determine the approximate percentage of the workload for resistance. In the end of the sessions, the stretching was performed individually, with emphasis in the upper and lower back, shoulders, arms, chest, abdomen, thighs and calves.

2.4. Control subjects

Thirty healthy patients were recruited from the study group at the Center of Physical Education at the Federal University of Santa Maria through a single-stage, using a random sampling. They were aged between 55 and 65 y, both sexes, with normal blood pressure and were free of diabetes mellitus, obesity, dyslipidemia, alcoholism, cigarette smoking, or chronic diseases. Moreover, they had received no pharmacological therapy during the month before the study and had no cardiovascular disease. No exercise intervention was assigned to the control subjects, and all of them signed the consent to take part in the study. Ten milliliters of peripheral blood were collected by venipuncture from each patient and used for the isolation of platelets and for obtaining serum.

2.5. Anthropometric characteristics and blood tests

We measured the height, body weight, and abdominal circumference in all subjects studied. The morning blood pressure was recorded between 7 and 9 a.m. after subjects had been in a relaxed state for at least 10 min. The blood was collected after 12 h of overnight fasting in tubes without an anticoagulant system and centrifuged at 1800 $\times g$ for 10 min. The precipitate was discarded, and the serum was used to determine the level of HDL, total cholesterol, triglycerides, and glucose using standard enzymatic methods with Ortho-Clinical Diagnostics® reagents on a fully automated analyzer (Vitros® 950 dry chemistry system; Johnson & Johnson). The level of LDL-cholesterol (mg/dl) was calculated by using the Friedewald formula: [total cholesterol (mg/dl) − [HDL-cholesterol (mg/dl) + (triglyceride (mg/dl) / 5)]]. Total blood with was used to determine the total platelet count and the mean platelet volume (MPV) with a Coulter-STKS analyzer.